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(54) MONOCLONAL ANTIBODY, HYBRID CELL, AND PRODUCTION OF MONOCLONAL ANTIBODY

(57)Abstract:
PROBLEM TO BE SOLVED: To produce both a monoclonal antibody specifically recognizing a formyldehydropiperidine structure possessed by an additive of acrolein to lysine which is one of constituent amino acids of a protein or a peptide and a hybrid cell, and to provide a method for producing the monoclonal antibody by paying attention to the additive.
SOLUTION: This monoclonal antibody is capable of specificlly recognizing a formyldehydropiperidine structure and is prepared by immunizing a warm-blooded animal with a protein modified with acrolein, preferably a protein composed of a keyhole limpet hemocyanin modified with the acrolein as an antigen, fusing the resultant cell capable of producing the antibody to a cell of myeloma, providing a hybrid cell and producing the monoclonal antibody capable of specifically recognizing the formyldehydroopiperidine structure from the hybrid cell.

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CLAIMS

[Claim(s)]
[Claim 1] The monoclonal antibody which recognizes formyl DEHIDORO piperidine structure specifically.
[Claim 2] Said monoclonal antibody is a monoclonal antibody given in claim 1 term which is made to unite the antibody forming cell and myeloma cell which are obtained from the homeotherm by which used as the antigen protein embellished with the acrolein, and immunity was carried out with this antigen, considers as a hybrid cell, and is obtained from this hybrid cell.
[Claim 3] Said antigen is a monoclonal antibody according to claim 2 which is the hemocyanin of key ANAKASAGAI embellished with the acrolein.
[Claim 4] The hybrid cell which can produce a monoclonal antibody according to claim 1, 2, or 3.
[Claim 5] (1) The manufacture approach of the monoclonal antibody which prepares as an antigen the protein embellished with the acrolein, is made to unite the antibody forming cell and myeloma cell which are obtained from the homeotherm which carried out immunity with (2) this antigen, considers as a hybrid cell, and is characterized by acquiring the monoclonal antibody which cultivates (3) this hybrid cell and recognizes formyl DEHIDORO piperidine structure specifically out of culture medium.
[Claim 6] Said antigen is the manufacture approach of the monoclonal antibody according to claim 5 characterized by being the hemocyanin of key ANAKASAGAI embellished with the acrolein.

[Translation done.]

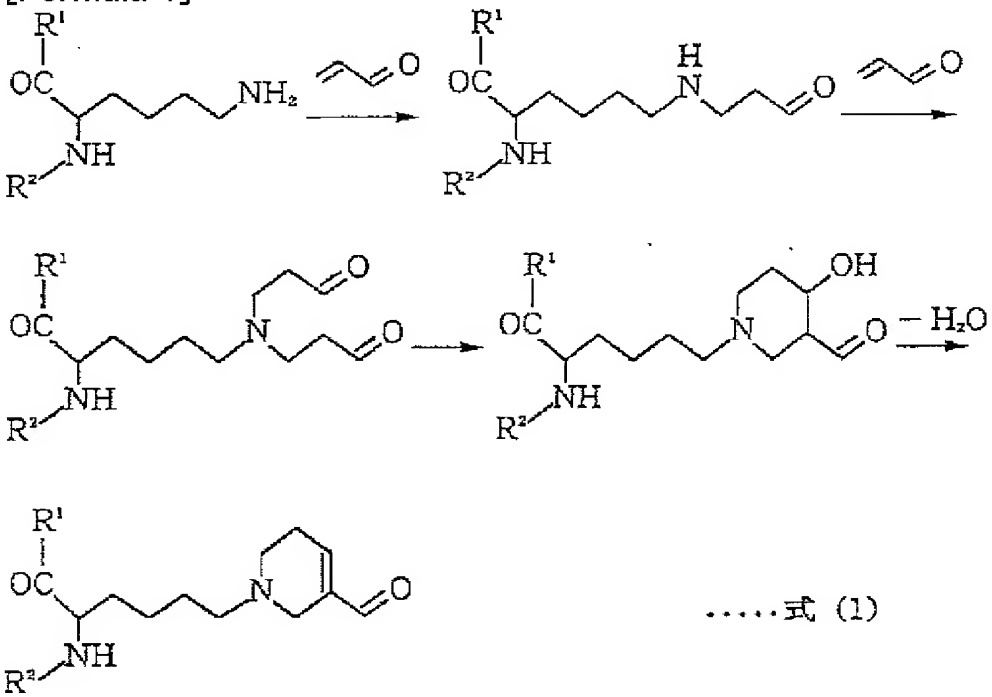
DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the monoclonal antibody which has the high singularity over the reactant of a monoclonal antibody and its manufacture approach especially an acrolein (code: ACR), and protein, and its manufacture approach. It is related with the monoclonal antibody which recognizes formyl DEHIDORO piperidine structure specifically, and its manufacture approach in more detail.
[0002]
[Description of the Prior Art] As a lipid component in the living body, a triglyceride, phospholipid, a cholesterol ester, or free fatty acid is known. When these compounds have altitude unsaturated-fat acid residue, it is shown clearly that this altitude unsaturated-fat acid residue receives peroxidation, peroxidation decomposition is carried out further, the peroxidation decomposition product of a lipid is generated by various oxidation stress which acts on a living body, and it is participating in various symptoms by it.
[0003] As a compound which has the above and altitude unsaturated-fat acid residue, linolic acid, a linolenic acid, an arachidonic acid, eicosapentaenoic acid, docosa-hexaenoic acid, etc. are typical. As the peroxidation decomposition product, aldehydes, such as a malondialdehyde, 4-hydroxy nonenal, and 4-hydroxy HEKISENARU, are known. These aldehydes react with protein in the living body, a peptide, or amino acid, and form an adduct, and the intervention with various diseases is studied briskly. It is checked that many adducts in an arteriosclerosis layer of a malondialdehyde or 4-hydroxy nonenal, and protein exist especially, and causal relation with arteriosclerosis attracts attention dramatically.
[0004] To a malondialdehyde, 4-hydroxy nonenal, or 4-hydroxy HEKISENARU generated from higher unsaturated fatty acid residue in the living body,

generally generating by combustion of heating of edible oil, a gasoline, or plastics is known, decomposition of the plastics used for the wrapping of edible oil or food etc. generates ACR, and it shifts in paths, such as taking orally, in the living body, and is apprehensive about various toxicity being shown to a living body. Moreover, in the latest research (Koji Uchida et al., Japan Society for Bioscience, Biotechnology and Agrochemistry, the collection of the convention lecture summaries in the 1997 fiscal year, No. 837, the 71st volume 53 pages), the report which shows generation of an acrolein in the living body is made, and causal relation with various symptoms is studied briskly. [H.Esterbauer which is what is depended on the property as a highly reactive aldehyde in which can consider carcinogenic etc. as living body toxicity which such ACR has, and ACR has this, H.Zollner and Z.N.Scholz, Naturforsch.30,466 (1975), J.C.Gan and G.A.S.Ansari, and Res.Commun.Chem.Pathol.Pharmacol. -- 419, 55 (1987), and]. Specifically, it is considered to be the cause with nucleophilic groups, such as an amine in which protein, a peptide, or amino acid in the living body etc. contains ACR, or a thiol, that reactivity is dramatically high. The reaction mechanism shown in a degree type (1) is known, and the compound which two ACR carries out an addition reaction to one amino group, and has formyl DEHIDORO piperidine structure generates the reaction of ACR and the amino group of amino acid.

[0005]

[Formula 1]



.....式 (1)

[0006] R1 and R2 show protein residue, peptide residue, or R1=R2=H among [type.]

[0007]
[Problem(s) to be Solved by the Invention] Thus, in relation to ACR from which correlation with a living body's disease attracts attention, it is dramatically important immunology or to perform biochemically the quality of an adduct (code: ACR adduct) with protein, a peptide, or amino acid or a quantum. Although the technique which makes these possible from a clinical-diagnosis study-or clinical medicine-standpoint is searched for strongly, the concrete means or the technique is not yet established.
[0008] Then, its attention was paid to this invention about the adduct of the lysine (code: Lys) which is one of ACR, protein, or the configuration amino acid of a peptide, and it tackled a monoclonal antibody with high recognition singularity, and development of that manufacture approach to the formyl DEHIDORO piperidine structure which this ACR adduct has.
[0009]
[The means for solving invention] As a result of this invention persons' inquiring wholeheartedly in view of the above-mentioned trouble, the hybrid cell of the antibody forming cell and mouse myeloma cell which are obtained from the mouse which prepared as an antigen the hemocyanin (code: KLH) of key ANAKASAGAI embellished with the acrolein (code: ACR), and carried out immunity with this antigen completed a header and this invention for producing the monoclonal antibody which recognizes formyl DEHIDORO piperidine structure specifically.
[0010] That is, the monoclonal antibody of this invention is characterized by recognizing formyl DEHIDORO piperidine structure specifically.
[0011] The hybrid cell of this invention is characterized by producing the monoclonal antibody which recognizes specifically the formyl DEHIDORO piperidine structure shown by the degree type (2).
[0012]
[Formula 2]

……式 (2)

[0013] R1 and R2 show protein residue, peptide residue, or R1=R2=H among [type.]
[0014] The manufacture approach of the monoclonal antibody of this invention prepares as an antigen the protein embellished with (1) ACR, unites the antibody forming cell and myeloma cell which are obtained from the homeotherm which carried out immunity with (2) this antigen, is used as a hybrid cell, and is characterized by acquiring the monoclonal antibody which

cultivates (3) this hybrid cell and recognizes formyl DEHIDORO piperidine structure specifically out of culture medium.

[0015] In said monoclonal antibody and its manufacture approach of this invention, it is desirable that the protein which should be embellished with ACR is the hemocyanin (code: KLH) of key ANAKASAGAI.

[0016]

[The gestalt of operation of this invention] The monoclonal antibody of this invention is manufactured by preparing the protein embellished with ACR, screening the antibody forming cell obtained from the homeotherm by which immunity was carried out by making this into an antigen, and the hybrid cell which prepares a hybrid cell with the myeloma cell in which subculture is possible for the obtained antibody forming cell, and produces only the target monoclonal antibody out of this hybrid cell, carrying out mass culture under the environment where this hybrid cell produces an antibody, and acquiring from a culture.

[0017] That the antigens which can be used for this invention should just be protein and the reactant of ACR, as protein, cow serum albumin (code: BSA), ovalbumin, lipoprotein, the hemocyanin (code: KLH) of key ANAKASAGAI, etc. are mentioned, and the hemocyanin of key ANAKASAGAI is used preferably, for example.

[0018] In the buffer solution, mixed stirring can be carried out and the protein for preparing an antigen and the reaction of ACR can be carried out. As the buffer solution to be used, tris, phosphate, a carbonate, and the acetate buffer solution are mentioned. as the buffer solution -- concentration -- 1mM-0.2M -- the phosphate buffer solution of 10-100mM is mentioned preferably. The terminal point of this reaction can be detected by the percentage reduction of the amino acid in protein. It asks for the percentage reduction of amino acid by measuring the amino acid content of the sample for example, before and behind a reaction with automatic amino-acid-analysis equipment (JEOLJLC-300, Jasco Corp. make). The reaction terminal point by amino acid percentage reduction is an event of the event of for example, lysine percentage reduction becoming 25% or more being mentioned, and decreasing 75% or more still more preferably 50% or more preferably. 1 - 72 hours is mentioned at 25-45 degrees C, and the reaction condition used as such lysine percentage reduction is 2 - 6 hours at 30-40 degrees C preferably.

[0019] The animal by which immunity is carried out by this invention can use a homeotherm. As this immune animal, especially if it is a mouse, a hamster, a rat, a guinea pig, a rabbit, a dog, a fowl, etc., for example, it will not be restricted, but since the myeloma cell which unites an antibody forming cell is the thing of the mouse origin, a mouse is used preferably.

[0020] The approach of carrying out immunity does not have 7, using the

usual well-known immunity approach, and will be desirable especially at intervals of 12 thru/or 16 days on the 30th. [of 2 or three administration] Although an antigen dose changes with animals by which immunity is carried out, let per [about] time (for example, about 0.05-2mg) be a rule of thumb. Although a route of administration can choose subcutaneous injection, intradermal injection, intraperitoneal injection, an intravenous injection, an intramuscular injection, etc., it is the administration gestalt performed by injecting a peritoneal cavity, hypodermically, or intramuscular preferably. It is the administration gestalt which combines the routes of administration of all 2 thru/or the route of administration combined three, for example, intraperitoneal injection, subcutaneous injections, and intramuscular injections for said route of administration still more preferably.

[0021] In addition, although an antigen can be dissolved and used for a sodium phosphate buffer solution, a physiological saline, etc. containing one sort of adjuvants usually used, such as the suitable buffer solution, for example, Freund's complete adjuvant, Freund's incomplete adjuvant, and an aluminum hydroxide, in this case, it is not necessary to use the above adjuvants. Here, an adjuvant means the matter which reinforces the immunoreaction to the antigen nonspecific, when a medicine is prescribed for the patient with an antigen.

[0022] After breeding without dealing with the homeotherm which carried out immunity of the above-mentioned antigen for seven - 30 days, little extraction of the blood serum of this homeotherm can be carried out, and antibody titer can be measured with the measuring method chosen from western blotting, a condensation method, enzyme immunoassay, single radial immunodiffusion, etc. in antibody titer. It can measure with enzyme immunoassay simpler. If antibody titer rises, according to a situation, count operation of the administration of an antigen of suitable will be carried out. For example, 0.01-1mg of 1 or two additional administration are preferably performed with the dose of a 0.05-0.5mg antigen. The organization containing the lymphocyte which produces an antibody 1 thru/or 30 days after the last administration from the homeotherm which carried out immunity one - seven days after preferably especially is extracted. Although any are satisfactory for it as long as the organization which extracts is the deletion lymphoid tissue containing the lymphocyte which produces an antibody, it is a spleen preferably.

[0023] By the approach indicated by "a guide to monoclonal antibody experiment operation information" (Kodansha scientific Ando **** s 1991) etc., in order that the obtained organization may consider as the cell in which subculture is possible, it can do cell fusion to a certain kind of gun cell under an Sendai virus or polyethylene-glycol existence, and can get a hybrid cell. Also as for the same homeotherm, it is desirable to use the gun cell of a

homeotherm of the same kind, for example, when uniting a mouse with the spleen cell obtained as an immune animal, as for the gun cell used here, it is desirable to use a mouse myeloma cell.

[0024] As the approach of cell fusion actually used, a well-known technique (308 J. Immunol.Method 39:285- 1980) can be used. For example, after uniting the splenic cells and mouse myeloma cell which were obtained from the mouse by which immunity was carried out under polyethylene-glycol existence, proliferating a hybrid cell selectively by the HAT medium (hypoxanthine, aminopterin, thymidine addition culture medium) which can grow only a hybrid cell and a hybrid cell's forming a colony, the hybrid cell which produces the target antibody can be obtained by screening the antibody in a culture supernatant.

[0025] As an approach of screening, western blotting or an enzyme immunochemistry-measuring method is mentioned, for example. Moreover, the hybrid cell which produces the target antibody can obtain a single hybrid cell eventually by repeating limiting dilution. Furthermore, the hybrid cell which produces the antibody of these objects can manufacture an antibody by carrying out mass culture under the environment which produces an antibody. Furthermore, the antibody which the hybrid cell which produces the antibody made into these objects produced can be refined by using the general biochemical separation approach about protein, such as the protein fractionation which uses centrifugal separation, an ammonium sulfate, or a polyethylene glycol, the aquosity bilayer distributing method, gel filtration chromatography, an ion exchange chromatography, affinity chromatography, and electrophoresis, combining the approach of independent or some.

[0026] The monoclonal antibody of this invention is applicable not only to detection of human ACR qualification protein but detection of the ACR qualification protein which exists in living bodies, such as homeotherms of a different kind other than Homo sapiens, for example, a mouse, a rat, a guinea pig, a rabbit, a dog, a cat, a cow, a horse, and a fowl.

[0027]

[Example] Hereafter, an example explains this invention concretely. In addition, this invention is not limited to this example.

[0028] [Example 1]

1. 37 degrees C of the hemocyanins (code: KLH) (2mg/(ml)) and ACR (10mM) of preparation key ANAKASAGAI of an antigen were made to react in 50mM phosphate buffer solution (pH7.2) 1ml for 24 hours. When the amino acid to which it decreased under ACR qualification KLH with automatic amino-acid-analysis equipment (JEOL JLC-300, Jasco Corp. make) was investigated, the lysine (code: Lys) was decreasing 80%.

[0029] 2. It often mixed with equivalent Freund's complete adjuvant, and immunity approach ACR qualification KLH (1mg/(ml)) was made into the

emulsion, and carried out this 100microl immunity into the peritoneal cavity of a mouse (BALB/c, a male, 6-8 weeks old). Ten - 14 days after the priming, Freund's incomplete adjuvant was often mixed with the antigen, it considered as the emulsion, and the booster was performed. After [of a booster] three weeks, phosphate buffered saline (code-BS) was mixed with the antigen, and the last immunity was performed. In addition, antibody titer checked that the antibody to an antigen was produced by enzyme immunochemical method using the blood serum which extracted blood and was obtained from the mouse eye socket vein after [of a booster] one week. In enzyme immunochemical method, between the blood serums obtained from the mouse in front of the immunity used as the blood serum obtained from the mouse which carried out immunity, and comparison contrast, the difference in comparatively big antibody titer was seen and lifting of antibody titer was checked.

[0030] 3. The symptom antibody titer of antibody titer was checked with the enzyme immunoassay which made ACR and cow serum albumin solid phase. That is, cow serum albumin (code: BSA) and ACR were made to react like the approach indicated to preparation of an antigen, and the ACR qualification BSA (code: ACR-BSA) was obtained. After it carried out physical adsorption of this to 96 hole immuno plate and 0.05%Tween20-tris buffers (pH7.4) (code: TTBS) washed 3 times, it blocked with the tris buffers (pH7.4) (it abbreviates to blocking liquid hereafter.) which contain BSA 1%. The blood serum (100microl / well) obtained from the mouse by the well of this plate was put in, and it was made to react at 37 degrees C for 1 hour. The liquid (100microl / well) (it abbreviates to an enzyme labelled antibody solution hereafter.) which diluted with TTBS the rabbit antibody to the mouse antibody by which the indicator is carried out by horseradish peroxidase 5000 times was put in after washing a well, and it was made to react at 37 degrees C for 1 hour. After washing a well 3 times by TTBS, the 0.1M citric-acid-phosphate buffer solution (pH5) (100microl / well) (it abbreviates to coloring liquid hereafter.) containing omicron-phenylenediamine (0.4mg/(ml)) and a hydrogen peroxide (0.003%) was put in, and it was made to react for 15 - 20 minutes at a room temperature. It stopped by putting in the sulfuric acid (50microl / well) (it abbreviating to the reaction stop solution hereafter.) of 1M, and the coloring reaction measured the absorbance of 492nm with the microplate reader, and checked antibody titer.

[0031] 4. The mouse spleen was extracted from the mouse in which syncytium carried out preparation immunity, it unfolded well, and splenic cells were obtained. The obtained splenic cells were washed by RPMI-1640 culture medium. It mixed with mouse 653 cell (P3X63-Ag8,653:CRL, 1580) which are these washed splenic cells and a myeloma cell often similarly

washed by RPMI-1640 culture medium so that the number of cells might become the rate of 7:1, and to the culture medium, 50 w/v% of polyethylene-glycol 1540 solution was added gradually, and it mixed with it for 5 minutes. After adding RPMI-1640 culture medium to this and stopping a reaction, at-long-intervals alignment separation was carried out for 5 minutes, and supernatant liquid was discarded. After adding RPMI-1640 culture medium to this, at-long-intervals alignment separation was performed for 5 minutes, and supernatant liquid was discarded. This actuation was repeated twice, the cell was washed and syncytium was prepared.

[0032] 5. the syncytium from which the cloning above-mentioned was obtained -- a 30ml HAT medium -- adding -- a cell -- suspending -- 96 hole immuno plate -- each -- 100microl **** distributive pouring was carried out at the well, and the hybrid cell was selectively proliferated by the HAT medium. ten days after fusion and HT culture medium (thing excluding aminopterin from the HAT medium) -- a well -- the culture supernatant of 1/2 inner amount was permuted. This actuation was repeated 2 to 3 times. screening concerning two antibody activity using this culture supernatant -- carrying out -- two screening -- antibody activity -- accepting -- ****** -- a well -- inner syncytium was sorted out as a desirable thing. The cell of the well by which antibody activity was checked cultivated by performing limiting dilution, and obtained seven shares of clones which have high antibody activity and consist of a single cell to ACR qualification protein by repeating screening and limiting dilution eventually. The mouse IgG subclass was examined about the 5F6 share monoclonal antibody which was strong among seven shares of obtained clones as for antibody activity. When the mouse IgG subclass assay kit (trade name: Mouse monoclonal antibody isotyping kit RPN29, Amersham make) examined the subclass of an antibody by making a culture supernatant into a sample, they were IgG1 and kappa (kappa) chain. When purification of an antibody was considered using a part of this culture supernatant, the monoclonal antibody of this invention was able to be refined by performing affinity chromatography which makes ligand the salting-out by the ammonium sulfate, or protein A as a conventional method.

[0033] 6. The antibody activity over the ACR qualification BSA was evaluated like the symptom of the antibody titer by enzyme immunochemical method, using as a sample the culture supernatant obtained with the screening approach -1 above about antibody activity.

[0034] 7. After mixing 0.5ml of LDL solutions of the -21mg/ml of the screening approaches and 0.1ml of copper-ion (Cu2+) solutions of 50microM about antibody activity and being referred to as 1ml with 50mM phosphate buffer solution (pH7.4), it incubated at 37 degrees C for 72 hours, and Oxidation LDL was prepared. it becomes in ml and 50microg /about the

acquired oxidation LDL -- as -- 50mM phosphate buffer solution -- diluting -- 96 hole immuno plate -- each -- physical adsorption of the 100microper well I was added and carried out. Next washing or a blocking process was performed like the symptom of the antibody titer by enzyme immunochemical method, and measured the antibody activity over Oxidation LDL as an absorbance in 492nm eventually.

[0035] [Example 2] It checked about the reaction singularity of a 5F6 share monoclonal antibody among the monoclonal antibodies which recognize the ACR qualification protein obtained from the seven-share clone obtained in said example 1. this -- the 5F6 share is deposited with National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, the Ministry of International Trade and Industry, as a deposition number (FERM P-16514) on November 11, Heisei 9. In addition, the monoclonal antibody used for examination diluted the culture supernatant, and it was used for it as it was.

[0036] 1. Enzyme immunoassay estimated the reaction singularity of the monoclonal antibody of appraisal method -1 this invention of the reaction singularity of a monoclonal antibody. That is, after having added the protein or aldehyde qualification protein (4microg/(ml)) of 100microper well I to 96 hole immuno plate, having put at 4 degrees C one whole day and night, carrying out physical adsorption to the plate and washing 3 times by TTBS of 300microper well I, the block ace (SNOW BRAND CO., LTD. make) who diluted with the BSA content TTBS or distilled water 4 times 1% was 300micro[per well] I Added and blocked. this monoclonal antibody solution (1microg/(ml)) diluted with TTBS of 100microper well I after washing this plate 3 times by TTBS like the above -- in addition, it incubated at 37 degrees C for 3 hours. In addition, only TTBS was added as control. After washing this plate 3 times by TTBS like the above, the enzyme labelled antibody solution of 100microper well I was added, and it incubated at 37 degrees C for 1 hour. After washing this plate 3 times by TTBS, the coloring liquid of 100microper well I was added, and it incubated for 15 - 20 minutes at the room temperature. After adding the reaction stop solution of 50microper well I, the absorbance of 492nm was measured with the microplate reader, and the reaction singularity of the monoclonal antibody of this invention was evaluated.

[0037] 2. Enzyme immunoassay estimated the reaction singularity of the monoclonal antibody of reaction singularity appraisal method -2 this invention of a monoclonal antibody. That is, to the well of the plate which added the ACR qualification BSA to 96 hole immuno plate, and carried out physical adsorption to the plate, except making into a sample what added the ACR derivative of various concentration to the monoclonal antibody (1microg/(ml)) by this invention, the absorbance was measured like the

approach of a publication for the preceding clause, and the reaction singularity of the monoclonal antibody of this invention was evaluated.
[0038] 3. the reaction singularity -1 of a monoclonal antibody -- the reaction singularity over various protein was evaluated about the monoclonal antibody of said example 1. Namely, ACR, a crotonaldehyde, 2-hexanal, 2-octenal, 2-nonenal, n-pro panhard, n-pentanal, n-hexanal, 4-hydroxy-2-pen TENARU, 4-hydroxy-2-hexenal, 4-hydroxy-2-octenal, 4-HIDOROSHIKI-2-nonenal, BSA embellished with aldehydes, such as a malondialdehyde, DL-glyceraldehyde, a hydroxy acetone, dihydroxyacetone, and a glyoxal, was prepared with the conventional method, and it measured by the reaction singularity appraisal method -1 of the aforementioned monoclonal antibody. The result was shown in drawing 1 as a graph with which the axis of ordinate showed the various aldehyde qualification BSA, and the axis of abscissa showed the absorbance (O. D.). Although BSA embellished with ACR has been recognized as shown in drawing 1 , BSA embellished with other aldehydes has not been recognized at all.
[0039] 4. the reaction singularity -2 of a monoclonal antibody -- the reaction singularity over various ACR derivatives was evaluated about the monoclonal antibody of said example 1. That is, the N-alpha-acetyl lysine, the N-alpha-acetyl-epsilon-formyl DEHIDORO piperidino lysine, the N-alpha-acetyl histidine, and the N-alpha-acetyl-pro panhard histidine were prepared according to the conventional method, and it measured by the reaction singularity appraisal method -2 of the aforementioned monoclonal antibody as contention matter. The result was shown in drawing 2 as a graph with which the axis of ordinate showed the rate of inhibition of the contention matter (B/B0: B and B0, in addition, show the absorbance under existence of the contention matter and nonexistence, respectively.), and the axis of abscissa showed the concentration of the contention matter. Only the N-alpha-acetyl-epsilon-formyl DEHIDORO piperidino lysine which has formyl DEHIDORO piperidine structure checked the reaction of the monoclonal antibody by this invention, and the solid-phase-ized ACR qualification BSA among the ACR derivative so that clearly from drawing 2 .
[0040] [Example 3] The 5F6 share monoclonal antibody was applied to immunity organization dyeing among the monoclonal antibodies which recognize the ACR qualification protein obtained from the seven-share clone obtained in said example 1. In addition, what refined the culture supernatant by the salting-out by the ammonium sulfate was used for the monoclonal antibody used for examination. Immunity organization dyeing was performed according to the approach generally used for immunity organization dyeing, for example, an approach given in "everything about staining techniques" (Ishiyaku Publishers , Inc. 155-165 1988), using the Homo sapiens arteriosclerosis focus as an organization which uses it for dyeing. That is,

after carrying out consecutive processing by the xylene, 50% ethanol, and 70% ethanol with the conventional method and obtaining a 3.5-micrometer organization intercept from the organization which did paraffin embedding, in order to perform activation nature of an antigen, it processed by the protease. Then, in order to prevent an endogenous peroxidase, it processed with the peroxidase blocking reagent S2001 (product made from DAKO). Then, in order to prevent a nonspecific reaction, it blocked by the rabbit normal serum diluted with phosphate buffered saline. The monoclonal antibody by this invention which exists in an organization intercept, and the part which reacts were observed by the ABC method (avidin-biotin complex method) which washes by making the phosphate buffered saline which contains the monoclonal antibody by this invention by 0.5-2.0microg [/ml] concentration react to this intercept at a room temperature for 1 hour, then uses a peroxidase as marker enzyme. In addition, diamino bench gin coloring kit 2V-0001-10 (Funakoshi Co., Ltd. make) was used for detection of a peroxidase. The microphotography (3; 200 times as many Olympus VANOX AHBS as this) which carried out immunity organization dyeing of the monoclonal antibody of this invention to a body tissue and the arteriosclerosis focus was shown as drawing 3 . The part dyed the Homo sapiens arteriosclerosis focus by drawing 3 by the monoclonal antibody of this invention so that clearly existed, and the positive stain remarkable in the intracellular organelle of a foam cell was accepted especially.

[0041] [The example 1 of reference]

1. After mixing 0.5ml of LDL solutions of -11mg/ml of usefulness of this monoclonal antibody, and 0.1ml of copper-ion (Cu2+) solutions of 50microM and being referred to as 1ml with 50mM phosphate buffer solution (pH7.4), it incubated at 37 degrees C for 72 hours, and Oxidation LDL was prepared. The enzyme immunoassay using the monoclonal antibody of this invention was measured by the reaction singularity appraisal method -1 of the aforementioned monoclonal antibody using this sample. The result was shown in drawing 4 as a graph which took the absorbance on the axis of ordinate and took incubation time amount on the axis of abscissa. (It displays by +Cu2in drawing 4 +)

[Example 1 of comparison reference] In addition, the reaction singularity of a monoclonal antibody was measured like the example 1 of reference except not using a copper-ion solution as an example of comparison reference. It was similarly shown in drawing 4 . (It displays by-Cu2in drawing 4 +)

[0042] According to drawing 4 , during the oxidization LDL by copper-ion processing, it is clear that the compound which has an ACR adduct, i.e., formyl DEHIDORO piperidine structure, exists, and it is useful to the research to ACR adduct analysis in a living body. By the system (- Cu2+) in which a copper ion does not exist on the other hand, Oxidization LDL does

not generate, but since the compound which has the ACR adduct of its origin, i.e., formyl DEHIDORO piperidine structure, does not exist, it is presumed that a monoclonal antibody does not react specifically.

[0043] [Examples 2 and 3 of reference] And [examples 2-4 of comparison reference]

2. 1Ml [Example 2 of Comparison Reference] of Glucose Solutions of Usefulness-210MM of this Monoclonal Antibody, 1ml [the example 4 of comparison reference] of ascorbic-acid solutions of glucose solution 1ml [example 3 of comparison reference] 20mM of 100mM(s), In addition, it incubated for one to four weeks at 37 degrees C so that it might become each solution of 1ml [the example 3 of reference] of 1:1 mixed liquor of 1ml [the example 2 of reference] of arachidonic-acid solutions of 20mM(s), 40mM arachidonic-acid solution, and a 200mM glucose solution in ml and 1mg /about cow serum albumin (BSA). The enzyme immunoassay using the monoclonal antibody of this invention was measured by the reaction singularity appraisal method -1 of the aforementioned monoclonal antibody using this sample. The result was shown in drawing 5 as a graph which took the absorbance on the axis of ordinate and took incubation time amount on the axis of abscissa.

[0044] According to drawing 5 , it is clear that the compound which has an ACR adduct, i.e., formyl DEHIDORO piperidine structure, exists only in an arachidonic-acid system. Although the ACR adduct generation from the arachidonic acid which is unsaturated fatty acid in the living body is not yet proved, in these researches, the monoclonal antibody of this invention is useful, and if ACR adduct generation will be proved from now on, the monoclonal antibody of this invention will become useful in the application.

[0045]

[Effect of the Invention] This invention can offer the monoclonal antibody which recognizes specifically the acrolein-lysine adduct which has formyl DEHIDORO piperidine structure so that clearly from the above explanation. The monoclonal antibody of this invention is dramatically useful in order to clarify effect to the living body of the acrolein which is one of the reactant high aldehydes, and being used in a clinical diagnosis, clinical pathology, analysis, etc. is expected.

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is the graph which shows the result measured by enzyme immunoassay about the reactivity of the monoclonal antibody of this invention to the qualification object which made BSA embellish various aldehydes.
[Drawing 2] It is the graph which shows the result measured by enzyme immunoassay about the recognition site of the monoclonal antibody of this invention.
[Drawing 3] It is the microphotography in which the result of having carried out immunity organization dyeing of the monoclonal antibody of this invention to a body tissue and the arteriosclerosis focus is shown.
[Drawing 4] It is the graph which shows the compatibility of the monoclonal antibody of this invention to Oxidation LDL.
[Drawing 5] It is the graph which shows the compatibility of the monoclonal antibody of this invention to an oxidation arachidonic acid.

[Translation done.]